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## Imidacloprid Bark Treatment for Hemlock Woolly Adelgid (*Adelges tsugae*)

Matthew D Quintero

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**Imidacloprid Bark Treatment  
for Hemlock Woolly Adelgid (*Adelges tsugae*)**

Matthew D. Quintero

Thesis submitted

to the Davis College of Agriculture, Natural Resources and Design

at West Virginia University

in partial fulfillment of the requirements for the degree of

Master of Science in

Forest Resources Management

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Morgantown, West Virginia

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hemlock woolly adelgid, IPM

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## Abstract

### Imidacloprid Bark Treatment for Hemlock Woolly Adelgid (*Adelges tsugae*)

Matthew D. Quintero

Since its discovery in the United States during the 1950s, Hemlock Woolly Adelgid (*Adelgis tsugae* Annand) has touched much of Eastern hemlock's (*Tsugae canadensis* L. Carrière) native range. Arborists have successfully handled this pest using either contact or systemic treatments. One of these treatments, imidacloprid, has often been used as a soil treatment. Due to its dependancy on water uptake by the tree, the insecticide sits in the soil for prolonged periods of time posing a threat to aquatic invertebrae and our drinking water. Imidacloprid has been labeled for use as a basal bark spray to control HWA, however limited information regarding its efficacy is available. Basal bark applications stand to serve technicians as a new tool by reducing translocation time, labor costs, material costs, eliminate tree wounding, minimize liability, and exposure to the environmen.

By comparing bark sprays to soil application this project will determine: 1.) if imidacloprid or olefin concentrations are similar between bark applications and soil application 2.) whether high or low labeled rates influence the concentration of imidacloprid or olefin compounds 3.) if bark applications are as effective as soil applications at reducing *A. tsugae* populations 4.) the neccessity of an adjuvant (Pentra-bark® ) and 5.) the feasibility of the application method. Determination was conducted by treating fifty-six trees by soil injection, basal bark application, or basal bark plus an adjuvant. These treatments were replicated at 0.67g/2.5cm DBH and 1.37g/cm DBH, the low and high labeled rates. This experiment, arranged in a 3x2 factorial fashion, also included a control.

Tissue concentrations of imidacloprid and olefin were analyzed by LC/MS/MS while biological efficacy was assessed through the collection of three population samples and the use of Abott's mortality correction formula. Orthognal contrasts were used for all analyses. Imidacloprid tissue results suggest no significant differences existed between soil and bark applications ( $P=0.0789$ ), bark applications and bark + Pentra-bark® applications ( $P=0.8358$ ), and low and high labeled rates ( $P=0.1181$ ). Olefin concentrations suggest no significant differences existed between soil and bark applications ( $P=0.2121$ ), bark applications and bark + Pentra-bark® applications ( $P=0.7721$ ), and low and high labeled rates ( $P=0.5013$ ). Biological efficacy results suggest that significant differences existed only between the bark applications and soil applications six months post treatment ( $P=0.0384$ ) but not at eleven months ( $P=0.0855$ ). Financial results indicate no significant difference was found between soil and bark applications ( $P=0.1194$ ) and between bark applications bark + Pentra-bark® applications ( $P=0.1009$ ), while significant differences existed between low and high labeled rates ( $P=0.0004$ ).

## **Dedication**



**In memory of Chris Colella.**

**1989-2015**

May your enthusiasm, curiosity, and passion for trees never be forgotten.



## **Acknowledgements**

Many people have graciously aided me along this project and I am thankful to all of them. I would like to begin with Dr. Michael Coyle to whom I owe much gratitude. Without his guidance from youth to adulthood, my life would be much different. It was through his constant encouragement and patience by which I returned to school and this project was conceived. My deepest thanks belong to my wife Elizabeth, who did not object to moving away from the home we made back to the Mid-Atlantic. Without her willingness to do so, this project would not be possible.

Throughout my nine years in the Army National Guard, I have made several friends that have become family. First Lieutenant Brian Elliot and Staff Sergeant Guercy Dominique are two of these, and without their appearance in the nick of time, my move to West Virginia University would have been waylaid.

While initiating this project, Aaron Dickinson of Rainbow Treecare Scientific, Mike Raupp of the University of Maryland, and Dan Gilren of Cornell University spent their valuable time thoughtfully answering my questions and providing guidance that pointed me in the right direction to developing a research topic. With a topic established, Biff Thompson of the Maryland Department of Conservation and Natural Resources aided me in the location of a suitable site.

With a project established, Dr. Elizabeth Benton of the University of Tennessee, Dr. Rick Turcotte of the U.S. Forest Service, Dr. Anthony Lagalante of Villanova University, and Dr. Richard Cowles of the Connecticut Agriculture station were all very kind in answering questions regarding imidacloprid despite being associated only by a common area of research. Dr.

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Throughout the entire process, my committee patiently guided me along through completing this degree. Dr. Gregory Dahle, Dr. Kathryn Gazal, and Dr. Yong-Lak Park of West Virginia University as well as Dr. Anand Persad of the Davey Research Institute not only shared their time, advice, attention, and expertise, but their resources as well. These resources made my time both in the field and in the lab easier and more enjoyable.

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## Chapter 1: Introduction

*Adelges tsugae* Annand (Hemiptera: Adelgidae), otherwise known as hemlock woolly adelgid or HWA for short, arrived on the East coast of the United States roughly seventy years ago. This invasive pest has killed enough eastern (*Tsuga canadensis* (L.) Carrière) and Carolina hemlocks (*Tsuga caroliniana* Englemann) for the trees to be declared as “near threatened”, only two levels above endangered, by the International Union for the Conservation of Nature (IUCN 2011). Due to the vast geographic range of its host species, land managers have had a hard time combating HWA.

Over the decades, a handful of chemical tools have slowly emerged making it easier for arborists and pesticide applicators to control adelgid on a limited scale. The interaction of a product’s chemical properties and federally approved application methods gives a product particular characteristics. These characteristics may make one product more favorable than another depending upon the environment in which applications are being made. With a limited number of products and application methods approved for HWA treatment, expanding either would benefit not only arborists, but land managers of all sorts.

The insecticide known as imidacloprid shows potential in providing arborists another way of combating HWA. It has been used in HWA treatments for several decades and its properties, that also make it the most popular pesticide in the world, may allow it to be applied in ways that have only recently been permitted (Elbert et al. 2008). First, it is systemic, meaning it can be internalized by a plant where it can lie to kill unsuspecting insects that feed upon that plant. Additionally, this insecticide possesses a long-lasting residue and is quite inexpensive.

These properties make it perfect for treating HWA; however the approved application methods are where it falls short.

Currently soil drenches, soil injections, trunk injections, and foliar sprays are the allowed methods. Soil injections or soil drenches both require equipment, such as spray rigs or specialized injectors. Trunk injections also require equipment and wound the tree. Likewise, foliar applications require spray rigs and heavy hoses that may limit how high they can reach. The fine mist produced from high pressure hoses creates the potential for chemical to drift off the intended target and perhaps onto adjacent properties. This unnecessarily exposes the environment to chemicals and the applicators liability.

Having used many products and application methods as a professional arborist, the frequency of HWA infestations left me with a need for a method that was easy to carry and quick to apply. Products applied to the bark meet these needs as they are transported and applied through a backpack sprayer, a conveniently carried apparatus. While imidacloprid is not labeled for bark applications, even for the treatment of other insects, it does belong to a class of chemical insecticides known as neonicotinoids, of which, several are labeled for basal bark applications.

Evidence suggests that basal bark applications may not only work, but technicians and chemical manufacturesrs are interested in this method as well. This may especially be the case in Nassau and Suffolk counties of New York where soil injections are prohibited. Such evidence includes a 2(ee) exemption made by the New York Department of Environmental Conservation for bark applications of particular imidacloprid formulations.

## *Research Objectives*

The overarching goal of this research project was to examine the plausibility of adding another tool by which pesticide technicians can combat HWA. This will be accomplished by determining:

- Whether imidacloprid can systemically penetrate bark on its own
- If a particular labeled rate is required to penetrate bark
- If an adjuvant is required to penetrate bark
- Imidacloprid's effectiveness against HWA.
- By performing a comparative financial analysis between imidacloprid bark applications and soil injections.

## *Hypothesis*

After reviewing pertinent literature, the most likely outcome of this experiment appears to be:

- imidacloprid will penetrate bark on its own
- adjuvant is not needed for bark penetration
- labeled rates will have an impact on penetration
- adelgid reduction will be comparable to soil injections
- the overall cost will be similar to that of soil injections



## Chapter 2: Literature Review

### A. Hemlock Distribution

Approximately ten species constitute the genus *Tsuga* within the *Pinaceae* family. The number of species classified as being within this genus has fluctuated over time (Dirr 1998; Havill, Vieira, and Salom 2014). Four of these are native to North America: Carolina hemlock (*Tsuga caroliniana*), eastern hemlock (*T. canadensis*), mountain hemlock (*T. mertensiana*), and western hemlock (*T. heterophylla*). Of these four, eastern and Carolina hemlock are native to the Eastern seaboard, while mountain and western hemlock reside on the Western seaboard (Joseph et al. 2011). Global distribution is illustrated in Figure 1.



Figure 1: Global distribution of all hemlock species. *T. canadensis*, the subject of our focus, resides on the North American Eastern Seaboard (Nathan Havill, USDA Forest Service, Northern Research Station). Over time, the number of species considered apart of this genus has fluctuated.

## *Eastern Hemlock*

As the most populous hemlock species of Eastern North America, *T. canadensis* will be the focus of this thesis. This slow growing and shade tolerant conifer is a frequent landscape tree in the Eastern U.S. urban forest (Webb et al. 2003). Maturity is reached between 250 and 300 years of age while some specimens have been found to reach an age of 900 years. In forest stands they may spend 25 - 400 years in the understory (Burns and Barbara 1990).

Trees are typically 30 m tall, with a broad and conical crown. The branches tend to drop at the ends causing a “feathery” appearance. Their needles are evergreen, about 20 mm long and flat. Needles’ top surfaces possesses a distinct green sheen while the bottom sports two broad white stomatal bands. Seed cones are roughly 2 cm long, ovoid, and hang near the branch tips. Cone production begins at about fifteen years of age (Guy Nesom 2000).

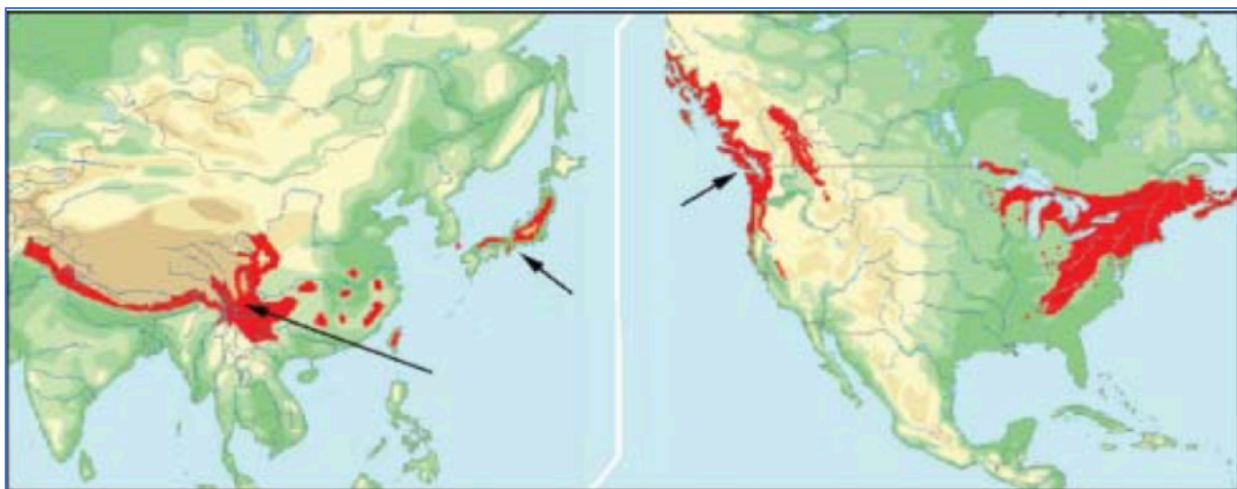
Ovulate and staminate cones of eastern hemlock are present on separate clusters of the same branch, making *T. canadensis* monoecious. Pollination occurs between late April and early June while fertilization takes roughly six weeks. Both pollen and seeds are wind dispersed. Dispersal typically occurs from mid - October through winter. Cones open in mid-October releasing seeds. Of all the conifers in the Eastern U.S., eastern hemlock produces the most cones. While this is the case, germination rates are estimated at less than 25% percent (Burns and Barbara 1990).

In the urban environment, eastern hemlock has been used as a specimen, screen, or group planting. Due to its crown density, hemlock is often sheared to form an evergreen hedge. Its popularity has brought on the production of several cultivars for dwarf, compact, and weeping qualities (Dirr 1998).

Eastern hemlock grows from Nova Scotia to Minnesota, South along the mountains to Alabama and Georgia. At roughly 1,000 feet in elevation it begins to appear and at 2,000 feet its presence is abundant. It prefers slopes and anywhere drainage is decent (Dirr 1998). Meanwhile Carolina hemlock occurs in isolated pockets in the states of Virginia, North Carolina, South Carolina, Tennessee, and Georgia (Burns and Barbara 1990).

## **B. Hemlock Woolly Adelgid**

HWA is an insect belonging to the order Hemiptera. Of all the hemipteran species, it is closely related to true aphids (Aphididae) and phylloxerans (Phylloxeridae), as it shares similar characteristics such as a complex life cycle et. al 2014). It resides on nine of the ten hemlock species worldwide; however, it is only a significant pest to the two species residing on North America's East Coast: eastern and Carolina hemlock (Figure 2). Prior to its discovery on these, it had been found across Japan, mainland Asia, and the North American West Coast (Havill et. al 2014).



*Figure 2: Global distribution of HWA (Chea et. al 2014). Arrows indicate native ranges. Native ranges of the insect coincide with the the species of hemlocks not found on North America's East Coast. DNA evidence suggests the HWA populations that reached the Northeast came from Japan and not North America's Pacific Coast.*

Its introduction to the Eastern seaboard can be traced to Richmond, Virginia, as museum specimens have been found here dating back to 1951. Arrival in Richmond is believed to be prior to this date and most likely directly from southern Japan, not from the American West Coast as DNA evidence suggests (McClure 1987; Havill et al. 2006). The global population of HWA is comprised of five genotypes: four native to Asia and one native to Western North America. Estimates state HWA roughly occupies half of the eastern hemlock range (Cheah et al. 2004). The expanse has risen North; however, as it reaches cooler climates the spread has slowed. It is believed that the lineage present in eastern North America is adapted to the lower elevations of Southern Japan and may lack a cold tolerance (McClure 1987; M. S. McClure and Cheah 2002). However, research is calling attention to a possible Northern progression due to climate change and the possibility this pest is adapting to the cold conditions in its new range (McClure and Cheah 2002).

### *Adelgid Life Cycle*

The HWA life cycle is complex, as it can reproduce both sexually and asexually, has winged and non-winged forms, can utilize multiple hosts, and possesses multiple generations in a single year. In its native region, HWA alternates between two hosts (*Tsuga sieboldii* and *Picea Torano*) where these two species coexist. Of these hosts, *T. sieboldii* is considered the primary, as it is the host on which sexual reproduction takes place. Between both hosts, five generations occur over the course of two years.

In North America, populations can survive solely on the alternate host: the hosts being *T. heterophylla* and *T. mertensiana* in the West and *T. canadensis* and *T. caroliniana* in the East. Thus, the populations here experience a truncated lifecycle. Each year, a winter and spring generation occur on the alternate host, the first is termed the “sistens” and the second is termed “progre diens”. These Latin terms mean “to pause” and “to proceed,” respectively, as the sistens, or winter generation, experience an aestivation period (Figure 3). This suspense of physical development is to protect itself from the hot summer temperatures and, therefore, the winter generation is much longer. In North America, the sistens generation develops from June through March, while the progre diens develops much more quickly between the months of March to June. Overlap of these generations is common due to the long period of egg laying and hatching. Ovisacs of the winter generation hold roughly 300 eggs, while the spring ovisacs hold around 20 to 75 (Chowdhury 2002).

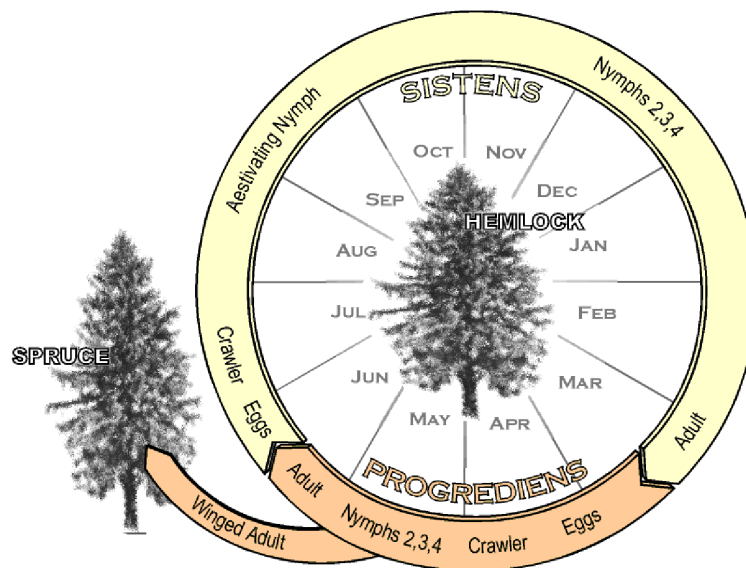


Figure 3: HWA Lifecycle. The lifecycle is quite complex as two generations occur each year. Such a lifecycle is described as being “bivoltine”, and consists of a progre diens and sistens generation, more commonly called the “spring/summer generations”. While this may be the case, generations are not well aligned with those seasons and overlap does occur. Figure from Ward et al. 2004

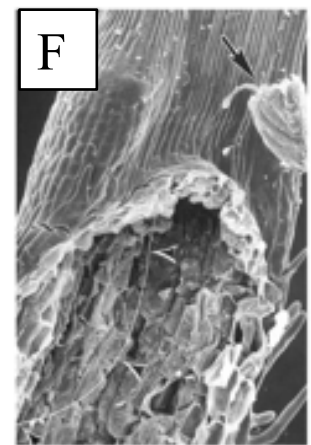
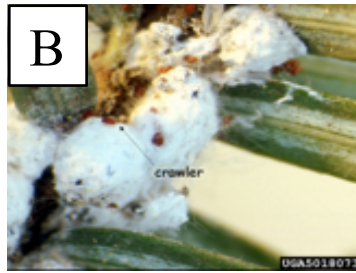
From the eggs (Figure 4A) of both generations, first instars known as crawlers emerge (Figures 4B & 4C). As the only mobile stage, crawlers search for suitable feeding sites on the underside of the needles. At a size of 0.3 mm they may be dispersed by wind or animals. These reddish-brown crawlers are also the most unprotected stage as they lack their “woolly” covering for which they are named. Once a crawler settles, it does so permanently as becoming a second instar (Figure 4D). At this point, the insect molts, changes from reddish brown, and becomes black, possessing a white waxy “halo” around its perimeter. It will retain these characteristics as it progresses into third and fourth instars (Figure 4E). When reaching maturity, a thick waxy woolly covering is formed (Figure 4F, 4G, & 4H). This covering is thought to serve as protection from desiccation and predators as it contains anthraquinones, biological compounds that serve as predator deterrents (Havil 2014, McClure 1987, Jones 2014).

Adults of the progrediens generation may be winged. These winged “sexuparae” (Figure 4I) consist of both sexes and fly in search of the primary host. Research of roughly a dozen spruce species in North America has found winged adults can find, colonize, and lay eggs in certain instances; however, the nymphs have not survived. Hence, it is considered that most of the individuals of HWA in North America are produced from asexual reproduction (McClure 1987).

In adelgids’ native region where a primary host can be found, another three generations flourish (Figure 5). Eggs laid by sexuparae produce a short sexual generation. Upon the conclusion of mating, females lay a single large egg that later develops into an asexual female, known as a fundatrix. Females overwinter in close proximity to where they feed upon the buds through the spring, forming a gall (Figure 4J). They lay many eggs that later hatch into crawlers, known as gallicole. Nymphal gallicole stages crawl into the gall where they feed until maturity.

They then emerge and fly back to the hemlock, drawing a conclusion to the five developmental stages and yearlong lifecycle on spruce (Cheah et al. 2004).





- A. Eggs
- B. 1<sup>st</sup> instar crawlers; note the size
- C. 1<sup>st</sup> instar crawlers; note the distinguishing color
- D. Nymphs (2<sup>nd</sup> instar) feeding at needle base
- E. 2<sup>nd</sup> instar nymphs lined along bottom of branch
- F. Needle cross section of adelgid feeding. The arrows point out the adult and the long stylet
- G. White woolly ovisacs give a cotton ball like appearance
- H. Mature adelgid with coiled stylet
- I. Winged sexupare
- J. Gall created by fundatrix on *P. Torano*

Figure 4: Various stages and anatomical features of HWA. See descriptions to right. Images I & J are courtesy of Nathan Havil, USFS. A-H are courtesy [www.forestryimages.org](http://www.forestryimages.org). Reference numbers can be found in lower right corner of the photo.



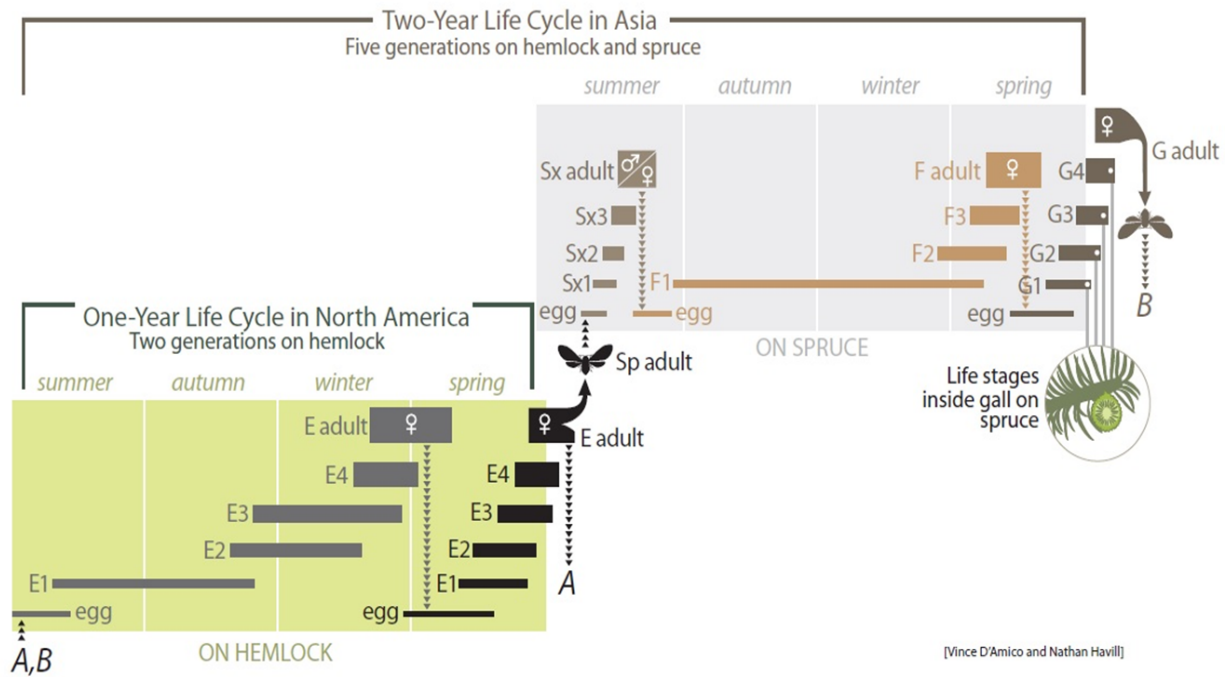


Figure 5: The presence of a suitable primary host in Asia means that the lifecycle is different there than that of North America (Nathan Havill, USDA Forest Service, Northern Research Station).

### Infestation & Damage Dynamics

Initially, HWA can be found on all ages of tree growth. However, the softer newest growth is more susceptible to infestation. It does not take many HWA to severely injure new shoots, reducing the production of future shoots. With fewer shoots in the second year of infestation, HWA mortality leaves populations on the older growth. As the third year progresses, the number of new shoots increases along with the HWA populations. With fewer shoots in year four, severe stunting of growth occurs. This phenomenon, by which the population ebbs and flows in a bimodal pattern, is known as density dependant feedback (McClure 1991; McClure 1987).

### *Feeding*

Upon hatching, crawlers search for a suitable site to feed. Presumably, they use bristles on their antenna, mouth, and legs as sensory organs. The insect settles at the base of needles on tender new growth where the waxy coating is thinnest, nutrient content is highest, and lignin is less abundant. Once settled, they use their stylets to feed. The long, thin, straw-like mouthpart penetrates epidermal cells, travels far along the vascular bundle between cells, and ultimately reaches and then taps into the xylem parenchyma cells where carbohydrates are stored (Preisser et al. 2014; Young et al. 1995).

While this seemingly small mouthpart is often thought of as one mouth piece, it is technically a bundle. Four individual stylets come together forming two canals. One canal can inject saliva while the other can draw nutrients from the tree. Saliva serves two suspected purposes: to carry bacteria for nutritional purposes and to harden around the stylet-bundle. The hardened shell allows of the removal and reinsertion of the stylet bundle as the insect ages and molts. Between removal and reinsertion, HWA will shed its cuticle. This process allows for feeding throughout the entirety of its life. (Havill et al. 2014; McClure and Cheah 2002; Young et al. 1995).

### *Damage Symptoms*

Feeding causes discoloration, desiccation, loss of needles, bud abortion, and branch dieback (McClure and Cheah 2002; Preisser et al. 2014). It appears there is no pattern to the distribution of HWA throughout tree canopies (Joseph et al. 2011). Often, effects can be visible after one year of infestation, while death can come in as little as three years. These figures are

highly dependent on environmental factors, such as soil moisture (McClure 1987; Joseph et al. 2011). Reports exist of trees lingering in a feeble state after fifteen years of infestation. Mortality rates as high as 90% have been reported in some stands, such as the Shenandoah National Park (Townsend and Rieske-Kinney 2006).

### *Damage Causes*

Research has suggested that the intense effect of *A. tsugae* upon eastern hemlock may not be solely caused by the consumption of parenchyma resources (Young et al. 1995). It is theorized “hypersensitive” reactions may be either a defensive response by which the tree kills nearby growth, thus limiting a food source or an induced response by which HWA increases local nutrient levels. In theory one, the killing of tissue causes false growth rings that interfere with water and solute transport, yet causes little harm to HWA and more harm to the tree. According to theory two, HWA may manipulate the plant through the secretion of enzymes. Amino acid concentration alterations have been found in HWA infested hemlocks. It is posed that this may be a similar process that occurs in galling insects (Preisser et al. 2014).

### **C. Biological Controls**

Integrated pest management (IPM) is a way that technicians handle pests of all types with various tools to avoid indiscriminate use of chemicals in the environment (Hokkanen 2015). Biological controls are just one option and, while perhaps more useful in forest management, they have made their way into the tree care industry as evident with other pests.

The first step in establishing such a control, is to identify predators in the native range of HWA. After identification, predators are studied in laboratories - where they are quarantined if outside their native range, reared, and finally released into the field (Cheah et al. 2004). Several predators have been identified and show promise, including three beetles from China (*Scymnus sinuanodulus*, *S. camptodromus*, and *S. ningshanensis*); a mite (*Diapterobates humeralis*); and two beetles, *Sasajiscymnus tsugae* - formerly known as *Pseudoscymnus tsugae* - and *Laricobius nigrinus* (Havill et al. 2014).

Many traits must be present to make a suitable predator, such as cold hardiness, life cycle synchronization between predator and prey, fecundity, and voraciousness. Therefore, some of these individuals have shown more promise than others in field trials. For example, the mite (*D. humeralis*) was identified in Japan, brought to the U.S. and studied, then finally released only to find it does not reproduce prolifically enough (Onken and Reardon 2011). Others, such as *L. nigrinus* and *S. tsugae*, have shown promise, although not without shortcomings. Currently research has begun investigating similar species (*L. rubidus* and *L. oakensis*) within the genus to discover a more suitable predator (Havill et al. 2014, Cheah et al. 2004). See Figure 6.



Figure 6: Potential biological control for HWA in North America. From left to right; *Sasajiscymnus tsugae*, *Scymnus ningshanensis* & *Laricobius nigrinus*. All are shown in adult stage. These have not yet been proven to be dependable enough to combat HWA in a way that chemical options can. Photos courtesy of [bugwood.org](http://bugwood.org). Reference numbers can be found in lower right corner.

Fungal pathogens are an area being explored as they too are environmentally friendly, although they are more expensive. Several are already used in the greenhouses of the horticulture industry. Pathogens such as *Beauveria bassiana*, *Metarhizium* spp., *Paecilomyces fumosorosus*, *Purpureocillium*, *Trichoderma* and *Lecanicillium* spp have all been successfully used there for several pests, including white flies and thrips. *L. spp.* are one of the more suitable fungi and are currently being sold and further explored. The pathogens of this genus attach to the exoskeleton, penetrate the cuticle wall, and then use hyphae to remove nutrients from the internal cavity. A commercial version of *L. muscarium* branded as Mycotal is distributed by Koppert B.V. Recent research shows that when released, this pathogen does not persist long enough to affect HWA (Wickert 2016).

#### **D. Imidacloprid**

##### *Alternate options and imidacloprid development*

Two insecticide types can be used for HWA treatment: horticultural oils and synthetic insecticides. Highly refined petroleum products, termed “horticultural oils”, work quite well on many insects regardless of their feeding type (Sunoco 1994). This is because the chemical properties allow for respiration and membrane disruption of the target insect (Dilling et al. 2009, Johnson 1985). It also happens to be one of the safest pesticides to both mammals and the environment. Oils have been used as a pesticide for over 100 years, well before the DDT era (Johnson 1985).

Oils are mixed with water and sprayed onto to plant foliage. Depending on the time of year, they are mixed at one or two percent to avoid burning foliage in hot weather. In the case of

HWA, application is through use of a hydraulic sprayer allowing arborists to reach canopy tops. It must be applied thoroughly around the tree and to the point of runoff (Rhea 1995). As there is no residual, timing is important. Ideally, applications are made to target adelgid at its most vulnerable life stages - the crawler or nymph stages - so that the treatment is most effective. Best management guidelines, provided by state extension services, often recommend three treatments a year (Lamb et al. 2011).

Besides oils, several insecticides belonging to the group known as neonicotinoids can be used for treatment. Dinotefuran and acetamiprid are approved for bark applications. Dinotefuran works rapidly and even treats elongate hemlock scale, another serious pest of eastern hemlock (Cowles 2010). Suppression of HWA is reported to be from 79% to 87% one month after application (Joseph et al. 2011). Acetamiprid trunk injections have been successfully used in HWA treatments. (Joseph et al. 2011, Persad unpublished). Other unpublished research into imidacloprid's use as a bark spray reports that of several neonicotinoids tested on ~3 m tall hemlocks, acetamiprid is one of the most readily absorbed and translocated (Cowles unpublished).

Of the four insecticide classes available in the 1980s - carbamates, pyrethroids, organophosphates, and pheno-pyrazoles - many pests had developed resistance to the first three (Simon-Delso et al. 2015). During this period, the compound imidacloprid was discovered by Professor Shinzo Kagabu while working for Shell Development Company. Soon after it was introduced onto the market in 1991 (Tomizawa and Casida 2010).

Its discovery and introduction ushered in the era of a new class of insecticides: neonicotinoids, synthetic compounds derived from the naturally occurring insecticide nicotine.

Since then, seven have been introduced: imidacloprid, thiacloprid, clothianidin, thiamethoxam, acetamiprid, nitenpyram, and dinotefuran (Jeschke 2011). By 2008, this new fifth class now constitutes a quarter of the insecticide market (Casida and Durkin 2013). Their popularity has grown because of their insecticidal control, low risk to non-target organisms, and systemic capabilities (Tomizawa and Casida 2010). Neonicotinoids happen to be the most effective insecticide for the control of piercing/sucking insects such as aphids, leafhoppers, and thrips.

### *Characteristics*

Merit®, the first branded formulation of imidacloprid came off patent in 2006. Since then, many other generic formulations have been produced. A few trade names include Xytect 75 WSP®, Touchstone®, and Advantage®. According to chemical nomenclature conventions, imidacloprid is known as 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine (Tomizawa and Casida 2003). It is considered a broad-spectrum pesticide, meaning it is effective against a wide variety of insects. As a neonicotinoid, it works as a contact spray and systemic application and therefore is a popular choice when handling *Adelgs tsugae*. The ability to move through the xylem is result of the high water solubility, low vapor pressure, water partition coefficient, and dissociation coefficients. Due to the mode of action, imidacloprid possesses a low to moderate mammalian toxicity and a class III to II toxicity rating (Lamb et al. 2011). The nature of its chemical composition makes it non-volatile, gives it a low potential for volatilizing from water as well as a low soil absorptioncoefficient, known as “K<sub>oc</sub>” ( Fossen 2006). This, in part, is why it has become one of the most common pesticides globally. Characteristic details can be found in Figure 8.

### *Mode of Action & Lethal Concentrations*

By binding to the post-synaptic nicotinic acetylcholine receptors of neurons, imidacloprid interferes with normal nerve impulse transmission. Normally acetylcholine, the major excitatory neurotransmitter, is bound to and then degraded by the inactivating enzyme acetylcholine esterase. Mimicking acetylcholine, imidacloprid heightens and then blocks the firing of the postsynaptic receptors. However, imidacloprid is not removed by acetylcholine esterase. This causes uncontrolled muscle reflexes, paralysis, and death (Tomizawa and Casida 2003).

Lethal concentrations, termed “ $LC_{50}$ ”, is the measure of the insecticidal concentration required to kill 50% of a sample population of a specific organism. Imidacloprid literature has attempted to establish links between the lethal concentration, HWA mortality, and effectiveness.

Laboratory experiments testing the link between concentration and HWA mortality do so by first removing an infested distal branch and dipping the cut end in a imidacloprid and water solution. HWA mortality is measured at a set number of days after initiation. Laboratory tests have calculated a  $LC_{50}$  of 300 ppb twenty days post treatment and  $LC_{50}$  of 242 ppb thirty days post treatment. Meanwhile, field tests have found the  $LC_{50}$  to be roughly 120 ppb (Cowles and Lagalante 2009, Eisenback et al. 2010).

Literature has also attempted to link concentration and adelgid to efficacy through a variety of measurements such as the appearance of tree health, new shoot length, or percentage of shoots infested (PSI). Jeffrey Fidgen, in developing a simple method of quantifying HWA populations based on PSI, established two thresholds: one based on aesthetics (10%) and another on damage (30%) (Fidgen et al. 2006). Research linking percentage of shoots infested to insecticide



concentrations found that a 0% PSI was associated with 211 ppb in branch tissue while a PSI >1% was 41 ppb. Trees with >30% PSI contained >27 ppb in their foliage (Eisenback et al. 2014). This means there is a range of efficacy associated with levels of concentrations.

### *Imidacloprid Metabolites*

The imidacloprid molecule is made of three important chemical groups: chloropyridinylmethl, imidazolidine, and nitroimine (Figure 7). When in solution, whether it be within the xylem or the soil, imidacloprid may metabolize, meaning the chemical groups comprising the parent compound may separate and recombine making new compounds, known as metabolites. This separation and recombination takes place as imidacloprid hydrolyzes, oxidizes, or isomerizes when in solution. Metabolites may be more or less lethal than the parent compound. Imidacloprid-olefin, or “olefin” for short, is just one such metabolite. With a 15 day

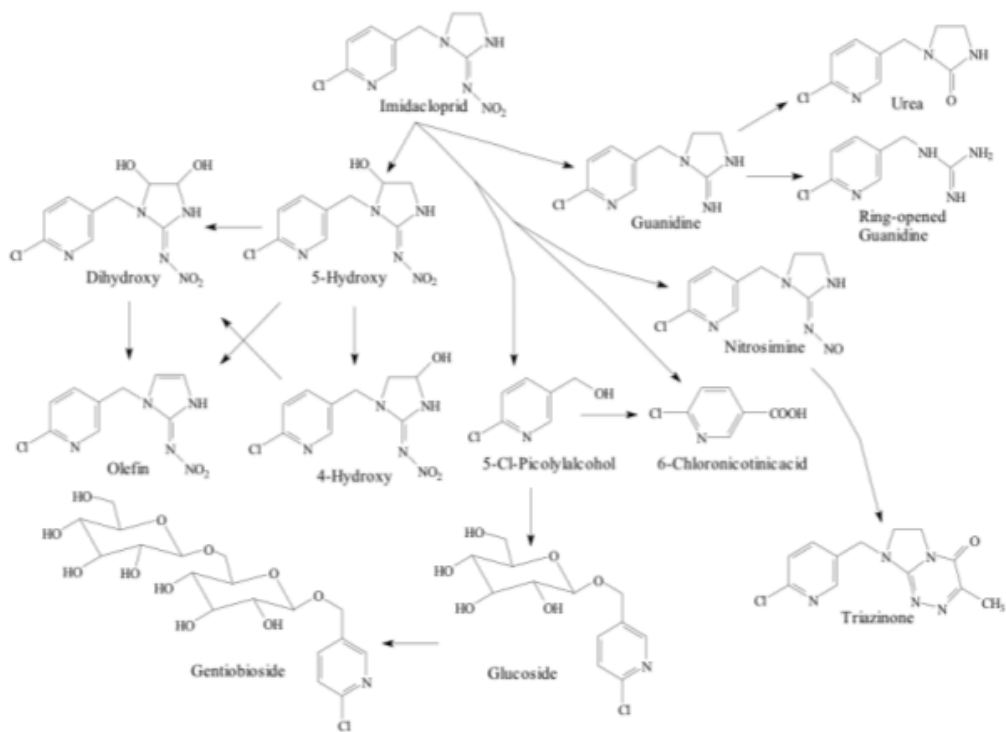


Figure 7: The parent compound and its metabolites are illustrated along with pathways in which they are formed (Placke & Gustin 1993). The reduction and recombination of imidacloprid into different compounds occurs as imidacloprid interacts with the environment where it may hydrolyze, oxidize, or isomerizes.

LC<sub>50</sub> of only 6 ppb, it has toxicity to HWA 10-16 times than that of the parent compound, imidacloprid. Olefin is believed to persist within the tree and provide it with a long periods of protection against adelgid (Benton et al. 2015). Metabolic pathways are illustrated in Figure 7.

### *Dosage*

Currently label instructions direct users to base application rates off of a tree's diameter at breast height. This linear relationship has been found to provide small trees with excess product and an inadequate amount in larger trees (Benton et al. 2016). Such a phenomenon is undesirable, as a per acre per year limit is enforced by the EPA/DEC, constraining how many trees can be treated at a given area and time.

Several projects have been aimed at correcting this. So far research has determined an exponential relationship between tree diameter and water usage. Additionally, it has linked water movement volume through xylem to imidacloprid concentrations. Tests of water use dosages proved to be more efficient than the current diameter method (Ford et al. 2010). Future research linking water usage to crown volume could make use of the findings by producing technicians friendly dosage tables for inclusion in the manufacturer's instructions (Turcotte et al. 2015).

### *Spatial Distribution*

The chemical properties discussed previously are what enable easy translaminar and xylem movement into shoots and leaves. Conversely, phloem movement to roots, fruits, and storage organs is poor (Elbert et al. 2008, Sur and Stork 2003). The older leaf portions can be

expected to house the highest residues (Sur and Stork 2003). Movement of imidacloprid throughout a single branch was studied by comparing the concentration levels between xylem fluid in the twig and needles. Of the samples tested, significant differences were found; 27% had detectable levels of imidacloprid in both xylem fluid and needle tissue, 14% had levels on in the xylem fluid and not in the leaf tissue, 23% were vice versa, and 36% had no detectable levels in either. This implies insecticide movement is intermittent (Turcotte et al. 2017).

Despite the many tests conducted over the years, researchers are still trying to grasp precisely why and where imidacloprid moves within the canopy. Studies have repeatedly examined this by dividing the crown of treated hemlocks into three strata: bottom, middle, and top. Some studies show mean concentration is highest in the lowest strata while others have determined it is highest in the middle and top strata (Coots et al. 2013, Dilling et al. 2009, Turcotte et al. 2017, E. Benton et al. 2016).

### *Temporal Distribution & Longevity*

Imidacloprid can be detected within three months of application, peaks between nine to fifteen months, and declines from there. Meanwhile olefin peaks around thirty-six months post treatment. Studies looking into the four to seven year window post treatment have still detected imidacloprid concentrations below the HWA  $LC_{50}$ . While this may be insufficient for adelgid mortality, it may contribute to adelgid suppression (Benton et al. 2015, Coots et al. 2013).

### *Environmental Fate*

In addition to being highly water soluble, imidacloprid poorly adheres to soil particles and is highly toxic to aquatic organisms. This can be of concern as eastern hemlock is naturally found in riparian areas, where the potential leaching of imidacloprid out of the soil and into surface water is possible. A similar concern exists in urban area where movement into groundwater is possible. (Fossen 2006 ).

Research shows imidicloprid's ability to adhere to soil particles rises with the amount of soil organic matter (Liu et al. 2006). The Connecticut Agriculture Station has addressed these concerns more specifically related to soil drenches for hemlocks. Tests found that imidacloprid and dinotefuran do not travel more than a few inches from where they are placed provided 3% organic matter is present (Cowles 2009).

Extensive research examining ground water contamination in New York State's Long Island region may corroborate Cowles' findings. In a region where sandy soils are dominant, the human population is dense, and the water tables are shallow, 46,316 applications of imidacloprid were recorded in two out of its four counties; Nassau and Suffolk. The New York Department of Environmental Conservation (NYDEC) has taken 2,000 samples from 11 upper water table monitoring wells across Suffolk county from 2001 - 2010 to find the majority of wells contained less than 1.0 g/L of imidacloprid. This is well below the NYDEC's maximum amount of 50 g/L, which is a standard eight times smaller than that of the Federal EPA standards (399 g/L) (Schatz 2017, NYDEC 2015). Provided it is applied to the soil in the correct conditions, when the soil is not dry or too saturated, imidacloprid can bind well to the soil. While Schatz does not provide

speculate why groundwater contamination is low when imidacloprid application is so prevalent, I suspect it is due to the chemical properties and adherence to better management practices.

In population dense urban areas, lingering of the product in the air would be another concern. A low vapor pressure and low Henry's law constant (Figure 8), mean it is unlikely to be present in the air in detectable amounts (Fossen 2006). While imidacloprid can be found in the soil and water, it does not persist long as imidacloprid is prone to photodegradation (EPA 2004).

Molecular weight	255.7
Water solubility	514 mg/L (20°C at pH 7)
Vapor pressure	$1.00 \times 10^{-7}$ mmHg (20°C)
Hydrolysis half-life	>30 days (25°C at pH 7)
Aqueous photolysis half-life	<1 hour (24°C at pH 7)
Anaerobic half-life	27.1 days
Aerobic half-life	997 days
Soil photolysis half-life	38.9 days
Field dissipation half-life	26.5 – 229 days
Henry's constant	$6.5 \times 10^{-11}$ atm m <sup>3</sup> /mole (20°C)
Octanol-water coefficient ( $K_{ow}$ )	3.7
Soil adsorption coefficient:	
$K_d$	0.956–4.18
$K_{oc}$	132–310

Figure 8: Specifications of characteristics related to environmental fate from Fossen, 2006. These chemical properties define imidacloprid's characteristics. At 514 mg/L, imidacloprid is moderately water soluble. It is by this ability imidacloprid can penetrate bark. With a low vapor pressure, it will not vaporize easily, meaning it won't linger in the air. With an aqueous photolysis half-life, imidacloprid will break down from the presence of light when in water. Its  $K_{oc}$  does not allow it to bind to soil particles easily.

### Non-target Organisms

Concerns of negative externalities include the insects of the forest ecosystem. Four hundred eighty-four arthropods have been associated with *Tsuga canadensis* (Turcotte 2015). Research exploring the effects of imidacloprid applications on arthropods is contradictory. Of the 484 arthropods investigated by Turcotte (2015), no difference in mean population levels were found when compared to the untreated control. Dilling (2009) conducted

similar research where he specifically examined the effects of various imidacloprid application methods and concentration levels on nontarget canopy insects associated with eastern hemlock, in particular phytophygus and transient insect communities. This accomplished by measuring specimen abundance, species richness, and guild abundance. Findings included: thirty-three nontarget canopy species were directly affected by treatments, application timing had no impact, and that higher concentrations of imidacloprid had effects on several of the metrics mentioned above. Several individual species are more sensitive to soil injections and drenches since they pupate in soil (Dilling et al. 2009).

Throughout the 1990s, arborists of the mid-Atlantic region observed high levels of mite infestations on hemlocks following imidacloprid application. Two studies investigating this issue concluding elevated mite populations are not present in every case of imidacloprid treated specimens (Raupp et al. 2004). The exact reason for this phenomenon is still unknown, however three common hypotheses include: sub - lethal doses stimulates mite reproduction, imidacloprid may eliminate or suppress mite predators, and imidacloprid alters hemlock physiology in a way beneficial to mites (Raupp et al. 2004). Treating for HWA and secondary outbreaks of mites, if necessary, is common practice amongst arborists.

Since 2007, Honey bees (*Apis* spp.) in the U.S. have been hit heavily by colony collapse disorder- sometimes referred to as CCD. Systemic neonicotinoids on the whole are a suspected cause. In the U.S. local, state, and federal decision makers are taking precautions to protect pollinators from neonicotinoids (Cowles 2010). It should be noted that the cause of CCD is still unknown (USDA 2012). On one hand, a review of the disorder around the world suggests several other explanations. On the other hand, imidacloprid has attributes that does not leave it

off the hook. Either way, hemlocks, like all pines, are wind pollinated meaning no effects to pollinators are anticipated.

<i>Daphnia magna</i> LC <sub>50</sub> (48 h)	85 mg/L
Mysid shrimp LC <sub>50</sub> (96 h)	37 mg/L
Rainbow trout LC <sub>50</sub> (96 h)	211 mg/L
Bobwhite quail LD <sub>50</sub>	152 mg/kg
Mallard duck LD <sub>50</sub>	283 mg/kg
Rat acute oral LD <sub>50</sub>	450 mg/kg
Honeybee LD <sub>50</sub> (48 h)	0.008 µg/bee

Figure 9: Toxicity ratings for various organisms (Fossen, 2006). Lethal concentrations or Lethal doses is the required amount of active ingredient needed to kill 50% of a test population after a specified amount of time.

### *Application Methods*

A variety of options exist, each having associated advantages and disadvantages. An arborist assesses each application method by their ease of application and off target potential. Treatment of hemlocks in the urban or residential sector often takes place in close proximity to people, pets, and structures, bringing the concept of off-target potential to the forefront of technician's minds.

Imidacloprid foliar sprays have found to be effective against adelgid populations (Cowles and Cheah 2002). When treating small trees or shrubs such a spray can be applied through use of a backpack sprayer while large trees require the use of larger hydraulic sprayers. In either case, just enough should be used to evenly coat the crown. When applied in this manner imidacloprid has rapid activity and a short residual (Silcox 2002). Tall trees or obstacles may preclude specimens from this method as hydraulic sprayers may not reach the treetops and may produce drift, increasing the risk of off-target damage (Figure 10).

Three types of soil treatments exist: soil drenches, soil injections, and tablets. Soil drenches (Figure 11A) are conducted by making a shallow trench around the base of the trunk and then pouring the mixture inside where the fluid is retained adjacent to the trunk. By contrast soil injections require equipment that allow the technicians to place the mixture below the surface and close to the roots, either around the dripline, in a grid within the dripline, or adjacent to the trunk (Rainbow Treecare Scientific 2012). Dosage is determined by DBH in both cases. Specialized soil injectors can be purchased (Figure 11B & C), or a more generic injector may be assembled rather easily. This can be done by simply plumbing a commonly available “fertilizing/root feeding gun” onto a spray hose of any portable container such as a backpack or a bottle sprayer as shown in Figure 11D & D. Tablets can be placed 5-10 cm below the soil, roughly 22 cm from the trunk or at the dripline when necessary. This treatment method places the active ingredient as well as fertilizer in the root zone for uptake (Bayer Environmental Science 2017).

Soil application methods have been proven to reduce HWA populations one to two years post treatment by 80% and 98% respectively (Cowles and Lagalante 2009). Compared to tablets research shows soil injections work slightly faster than tablets. Injections have produced lethal concentrations (>120 ppb) to HWA in as little as three months. Tablets took 12 months to cross this threshold (Cowles and Lagalante 2009, Eisenback et al. 2014).



### *Application Timing*

Several projects investigating whether spring applications or fall applications yields a higher imidacloprid concentration have produced conflicting results (Turcotte et al. 2017, Coots et al. 2013, Eisenback et al. 2014, Cowles and Lagalante 2009). Two of these studies report higher concentrations associated with spring applications, while the others found no difference among seasons. While this has not been reconciled, Carla Coots poses this may be due to regional differences, as the hemlocks of the Southern Appalachians transpire year round, unlike those of the Northeast (Coots et al. 2013).



*Figure 10: The limitations of spray equipment. Photo A shows the author using a bucket truck to treat ~21m hemlocks with horticultural oil. Photo B shows the author spraying lindens ~ 16m tall where drift is clearly depicted. Photo C too illustrates how much sprays drift through the use of a purple dye. This drift is highly undesirable for technicians in dense urban areas such as in photo A which was taken at a large home with construction taking place at time of application. Coordination with the homeowner and construction crew was required to clear the area of people. Other applications types such as soil and bark applications could avoid this. Photo C is courtesy of bugwood.org*



Figure 11: A: soil drench. B: self-calibrating injector. C: Kioritz Soil Injector.. D: backpack & soil injector E: bottle sprayer & injector. The equipment shown runs a wide range of prices and arborists may possess some of the pieces. The metal injectors pictured in D & E can be easily assembled with parts from a hardware store and are also used for sub-surface injections of fertilizer. Pictures B & D courtesy of Rainbow Treecare Scientific, C of [bugwood.org](http://bugwood.org).



There are a handful of trunk injection techniques including the Wedgel Direct Injection System, Mauget microcapsules, and the family of systems by Arborjet (Figure 12). While all these systems operate in a slightly different fashion, they function on the same basic principles; product is fed directly into the xylem by tapping the tissue with a drill, plug, or needle. With the xylem tissue tapped, pressure is supplied in some manner.



Figure 12: From left to left to right; Mauget Micro Injection Capsules, Arborjet "Viper System", & the Wedgel Direct Injection method. Photos Courtesy of [www.forestryimagery.org](http://www.forestryimagery.org), Arborjet, and Arborsystems™ respectively. The options are not limited to these three systems and like all professional equipment, these run a range of prices and are all slightly different in how they operate.

Research has produced varying results in regard to these injections. Work done by the Connecticut Agricultural station shows that a variety of these trunk methods showed no reduction in adelgid populations compared to untreated controls. Twig concentration showed that all of the tested trunk injection methods – Arborjet, Mauget, and Wedgel- Mauget was the only one that provided high levels of imidacloprid (240 ppb). Even in the case of Mauget, these high levels happened to only be peaks that diminished in a matter of months. And in all cases of trunk injections, no adelgid population control was achieved. The authors pose these results were due to uneven distribution throughout the canopy. Compared to the soil injections methods tested, the agricultural station found soil injections provided higher concentrations and a more uniform distribution throughout the canopy (Cowles and Lagalante 2009).

Similar research strictly comparing Maugé trunk injections to soil injections found that there was no difference in the adelgid populations or the number of shoots infested by those populations in the soil and trunk injections. The author states the injection method was on par with soil injections as it not only reduced the adelgid population but the number of shoots infested by the population as well. Differences were found in how the imidacloprid concentrations changed in the two treatments. Concentrations in the trunk injections appeared more quickly, reached a higher peak, and dissipated more quickly than the soil injections. The suggests the cause of the disparity between the Connecticut study and his own is due to differences in how trunk injections were performed (Eisenback et al. 2014).

### *Bark Applications*

Bark applications have been researched since at least the 1960s (Norris 1967, Pielou 1961). Other chemicals have been applied in this manner on what appears to be a handful of hosts/pests. This method stands to provide several benefits over other application methods. Early research shows depending on the product sprayed, bark applications could require as little as 1/10-1/5<sup>th</sup> the amount of active ingredient required in soil applications to attain the same levels of concentrations. Literature also proposes that they are not as dependant on water uptake compared to the soil applications (Norris 1967). It only follows that water could be carried more conveniently. Less product applied in a precise manner would eliminate the possibility of chemical drift while reducing environmental exposure when compared to foliar and soil applications. Drift elimination means reducing the concen of chemicals moving onto adjacent properties where children, pets, or valuable property may be screened by obstacles. This is

important to technicians as there are legal ramifications as state and federal agencies have laws to discourage this. More efficient use of the product means less sits in the soil where it may leach into waterways. This could be beneficial in forestes where eastern hemlock is often found in riparian settings. It stands that bark application may be easier to haul, cheaper to apply, friendlier to the environment, while eliminating tree wounds. The method is pictured in Figure 13.



*Figure 13: Bark applications are made using a backpack sprayer. Pressure is created by pumping the handle in the technician's left hand. When the handle on the wand is squeezed, the insecticide is released. It is applied from the technician's breast height down to the ground and is done to all sides of the trunk. The photo is to showcase the equipment and is not of an actual treatment, hence the lack of protective equipment.*

### *Adjuvants & Bark Penetration*

Pentra-Bark® is a surfactant and is used in combination with water soluble pesticides of all sorts. Marketing material states that when incorporated into the water/pesticide mixture, Pentra-Bark® proposedly opens bark lenticels to allow for pesticide movement directly into xylem tissue (Pentra-Bark 2017). When used with noenicitinoids, there is little evidence it provides the desired effect. Two studies examining basal bark sprays of noenicitinoids found that

Pentra-Bark® did not have any affect on mortality (McCullough et al. 2011; Cowles 2010).

Conversely, it has been found to make a difference on phosphite treatments of coast live oak for sudden oak death (Garbelotto et al. 2007).

While adjuvants are supposed to aid in penetration, penetration of the bark can occur without it through a process known as “active entry”. This phenomenon is the method by which water soluble pesticides are absorbed through cell walls of the periderm, into the cortical parenchyma and finally, into the phloem and xylem by cell to cell passage. This does not mean systemics can pass bark of all species as thick bark of mature trees may be impermeable. In the early years of experimentation, results show the larger the area to which a product is applied, the greater the degree of insect mortality is observed (Norris 1967).

#### *Detection in Tissue*

The first detection method is through the use of an enzyme-linked immunosorbent assay, abbreviated “ELISA”. This method used for testing xylem fluid is inexpensive but can result in falsely elevated concentrations of imidacloprid. This is due to cross-reactions between several of the metabolites associated with imidacloprid (Turcotte et al. 2017; Lagalante 2007).

Gas chromatography with positive chemical ionization mass spectrometry, also known as GC-PCI/MS, confirmed the cross reactions occurring with ELISA. This method was developed to overcome these issues and is used for detection in both xylem fluid (Lagalante and Greenbacker 2007).

Flow injection analysis with nitric oxide chemiluminescence detection (FIA NOCL) may be used on xylem fluid and needles. Detection is through the degradation of imidacloprid to its metabolites. Cross reaction of metabolites is also an issue in this method (Lagalante 2007; Lagalante and Greenbacker 2007).

Liquid chromatography tandem mass spectrometry, LC/MS/MS, can also be used for testing xylem fluid and needles. This technique does not suffer from inflated concentrations as other methods due and is able to detect metabolites in addition to the parent compound (Lagalante 2007, Turcotte et al. 2017).

## Chapter 3: Comparison of Biological & Financial Efficacy of Bark Treatments

### Introduction

Eastern hemlock (*Tsugae canadensis* Annand) inhabits much of North America's Eastern Seaboard. Preferring slopes and decent drainage, it can be found from Nova Scotia to Minnesota, then south along the Appalachian Mountains to Alabama and Georgia (Dirr 1998). Meanwhile, Carolina hemlock can be found in isolated areas throughout the Southeast, making it limited in number (Burns and Barbara 1990). The more populous eastern hemlock, the focus of this paper, has been used as a specimen, screen, or group planting throughout urban areas. Its popularity has brought on the production of several cultivars for dwarf, compact, and weeping qualities (Dirr 1998).

Much of eastern hemlock's native range has been effected since the 1950 discovery of hemlock woolly adelgid, *Adelges tsugae* L. Carrière (Hemiptera: Adelgidae), in the United States. DNA evidence suggests hemlock woolly adelgid, or HWA, arrived prior to this date from Southern Japan and did not migrate from the North American West Coast where this pest is also native (McFlure 1987; Havill et al. 2006).

HWA can be found on all ages of tree growth following initial infestation, although soft new growth is the most susceptible. It does not take many to injure new shoots, reducing the production of future shoots. With fewer shoots in the second year of infestation, mortality leaves populations on older growth. As the third year progresses the number of new shoots increases, as does the HWA population, bringing about fewer shoots in year four, where stunting of growth



occurs. This ebb and flow of the population is known as density dependant feedback (McClure 1991; M. McClure 1987).

Over the decades, arborists have handled this pest using several insecticides and application methods. The interaction of an insecticide's chemical properties and federally approved application method gives each product unique characteristics. Such characteristics may make one product more favorable than another depending upon the environment where applications are being made. With a handful of products and methods for HWA, treatment means expanding either would benefit arborists and land managers. Highly refined petroleum products, termed "horticultural oils", are one product that work quite well on many types of insects regardless of their feeding type (Suonoco 1994). This is due to how it affects insect respiration and their membranes (Dilling et al. 2009, Johnson 1985). It happens to be one of the safest pesticides to mammals and the environment and has been used for many pests over a century (Johnson 1985).

Of the four insecticide classes available in the 1980s - carbamates, pyrethroids, organophosphates, and pheno-pyrazoles - many pests had developed resistance to the first three (Simon-Delso et al. 2015). During this period, the compound imidacloprid was discovered and introduced to the market in 1991 (Tomizawa and Casida 2010). This ushered in the era of neonicotinoids, a class of synthetic compounds derived from the naturally occurring insecticide nicotine. Since then, seven more neonicotinoids have been introduced (Jeschke 2011). By 2008, this new fifth class constituted for a quarter of the insecticide market (Casida and Durkin 2013).

As a neonicotinoid, imidacloprid works as a contact spray and systemic application and therefore is a popular choice when handling HWA. The ability to move through the xylem is

result of the high water solubility, low vapor pressure, water partition coefficient, and dissociation coefficients. Due to the mode of action, imidacloprid possesses a low to moderate mammalian toxicity and a class III to II toxicity rating (Lamb et al. 2011). Its lack of volatility gives it a low potential for volatilizing from water and a low soil absorption coefficient, known as “ $K_{oc}$ ” (Fossen 2006). This in part is why it has become one of the most common pesticides globally.

Currently soil drenches, soil injections, tablets, and foliar sprays are the approved methods of applying imidacloprid for HWA control. Soil injections and soil drenches both require some equipment, such as spray rigs or specialized injectors while tablets don’t require any. Foliar applications require specialized equipment. The spray rigs and heavy hoses that are used limit how high a treatment can reach in addition to creating chemical drift. This fine mist lands off the intended target and perhaps onto adjacent properties. This unnecessarily exposes the environment to chemicals and the applicators to potential liability.

Professional arborists might prefer a less cumbersome and quicker method for treating HWA. Bark applications certainly meet these criteria and have been investigated at least as far back as the 1960s. Other pesticides have been approved by the EPA for use in this manner including Agri-fos® for sudden oak death (*Phytophthora ramorum*), Safari® for elongate hemlock scale (*Fiorinia externa*), and Baseline® for bark beetles (*Dendroctonus spp.*). This method stands to provide several benefits over other application methods. Early research shows depending on the product sprayed, bark applications could require as little as 1/10-1/5<sup>th</sup> the amount of active ingredient required in soil applications to attain the same levels of concentrations (Norris 1967, Pielou 1961).

Basal bark applications stand to reduce translocation time, labor costs, material costs, and minimize the exposure to the environment. By comparing treatments of imidacloprid as a bark spray to soil application this project will determine: 1.) if bark applications produce any imidacloprid or olefin concentrations 2.) whether labeled rates influence detectable levels of either compound 3.) the necessity of an adjuvant 4.) if bark applications reduce *A. tsugae* populations 5.) the feasibility of such an application. These comparative results will indicate whether this method can serve as another tool in the integrated pest management toolbox.

## **Methodology**

Research was conducted at Fallingwater, a Western Pennsylvania Conservancy property located in Mill Run, Pennsylvania. All trees lined the Bear Run Trail. The surrounding area was comprised primarily of silt loams, slopes between 35% and 70%, and is extremely stony (NRCS 2017). Prior to field treatment, 56 trees meeting basic criteria were identified, flagged, and numbered. Criteria included species, height, and spacing. Trees selected were eastern hemlocks, between 1.5 m and 11.2 m tall, and no closer than 9 m. The height requirement was to ensure all canopies could be accessed while the minimum distance between trees was to reduce the possibility of movement of product through the soil from tree to tree. Live Crown Ratio (LCR) and diameter at breast height (DBH) was measured at this time to gauge whether these cofactors influenced insecticide concentrations.

Once identified, trees were randomly assigned to receive one of seven treatments; six with imidacloprid and a seventh untreated control (Table 1). These imidacloprid treatments included a soil injection at the low labeled rate, soil injection at the high labeled rate, bark

application at the low labeled rate, bark application at the high labeled rate, bark application at the low labeled rate plus an adjuvant, and a bark application at the high labeled rate plus an adjuvant. The adjuvant rate was held constant in the middle of the labeled range (5.6 mL/4.5L). Eight trees were treated in each of the seven ways listed (totaling forty-eight treated trees and eight untreated trees).

*Table 1: Treatment types & dosages. \*Adjuvant was mixed at 5.6 mL of adjuvant for 4.5L of mixture in both groups where adjuvant was used. The upper and lower limits of the lower range, the range prescribed for “adelgids”, listed on the Xytect WSP label were used.*

Treatment Received	g AI/2.5cm DBH
Untreated Control (UTC)	0.00
Soil Drench- Low	0.67
Soil Drench- High	1.37
Bark Application- Low	0.67
Bark Application- High	1.37
Bark Application + Adjuvant- Low*	0.67
Bark Application + Adjuvant- High*	1.37

### *Field Treatment*

Soil injections were made through use of soil injector and bottle-pump (SPS Systems International, Santa Monica, CA.), while bark applications were applied through the use of a backpack sprayer (Greenwood™ Camrillo, CA.). Equipment used is featured in Figure 14. No calibration was required for the bottle pump as the water level inside the container was visible, volume markings were clearly marked, and the container was in front of the technician during use. Due to the nature of the backpack sprayer, calibration was required as it rested on the

technician's back. Calibration was performed between each treatments by averaging the time it took to fill a 0.24-L container three times.



*Figure 14: Application equipment used. Backpack and bottle sprayer are pictured in the rear. The soil injector laying across the foreground.*

Xytect 75 WSP® (Rainbow Treecare Scientific) and Pentra-bark® (Agbio, Inc.) were mixed in the containers and used for the insecticide treatments. Enough product was mixed to treat the eight trees determined to receive that particular treatment. Products were supplied by Rainbow Treecare Scientific and dosages can be found in Table 1.

Applications were made on May 12-13, 2017. With DBH measured prior to treatment, the required product amount for each tree was already calculated. Two technicians were used; one to operate the backpack sprayer and another to track the time it took to apply the product to an individual tree. Through calculating the volume of product required for each tree and calibrating the equipment prior to treatment, how long a technician should be pumping the

sprayer and applying product to the tree was known. Despite calibration, the back-sprayers did not maintain calibration well as some product remained at the end of each treatment. This remainder was divided evenly among the eight trees that received the same treatment.

### *Hemlock Tissue Preparation & Analysis*

Tissue samples were collected simultaneously with the population samples in November of 2017. Pole pruners were used to collect four samples from the tips of branches growing in the middle quadrant of the canopy. Branches from each tree were pooled in the bagging process and transferred to the lab on ice. The samples were transferred to paper bags, placed under a black bag and left in the dark to air dry for one week at 23.8 °C. Once dried, the needles were ground using a coffee grinder (Mr. Coffee™, Rye NY). UTC specimens were processed first, followed by the bark applications, and lastly soil applications. 99% Isopropyl alcohol Kimwipes® were used to clean the grinder after each sample processed. 1.0 g of needle grindings was measured (Mettler Toledo, Zürich Switzerland) placed into 15 mL centrifuge tubes, labeled, kept in dark/dry conditions, and sent to Villanova University for processing.

To extract the imidacloprid and olefin, a 1:10 (needle:solvent) ratio of pulverized needle tissue (1 g) and acetonitrile (10 mL) were mixed in centrifuge tubes (15 mL), and shaken overnight on an orbital bench shaker (Model G33; New Brunswick Scientific, Edison, NJ). The supernatant acetonitrile was analyzed for imidacloprid and its metabolites by liquid chromatography tandem-mass spectrometry (LC/MS/MS).

Imidacloprid and olefin quantification were conducted with a HPLC system comprised of binary Shimadzu LC-20AD pumps and a SIL-20A auto-sampler (Shimadzu, Columbia, MD).

Analyst software controlled HPLC separation (Applied BioSystems/SCIEX, Framingham, MA) run through a 2-mm Phenomenex Genini NX 2 mm guard column. An aqueous phase and organic mobile phase - water with 10-mM ammonium formate and acetonitrile - were used at a flow rate of 1.0 mL/min. A gradient elution ramped from 25 to 95% acetonitrile lasting one to eight minutes, used a column wash with 95% acetonitrile lasting eight minutes, and prior to the next injection performed a column stabilization period with 25% methanol. 10  $\mu$ L injections of imidacloprid and olefin standards were used for LC/MS/MS analysis.

A BioSystems/SCIEX 3200 Q - TRAP triple quadrupole mass spectrometer (Framingham, MA) was used to complete mass spectroscopy operating in positive electrospray ionization mode (ESI). Table 2 summarizes analytical MS precursor and fragment ions as well as the sensitivity of the LC/MS/MS method. Compound standards, outlined by Cook (2008), were analyzed in the ranges provided there. Standards were used to optimize multiple reaction monitoring transitions. Optimized electrospray ionization (ESI) source parameters were: curtain air gas 35 psi, Collision activated dissociation gas medium, ESI nebulizer gas 60 psi, auxiliary gas 60 psi, ESI probe temperature 550 C, and ion spray voltage 5500V. Collision cell exit potential was set to 4V and Q0 entrance potential to 10V. Q1 and Q3 resolution was set to high and dwell times for each transition was 500 ms.

The limit of detection (LOD) for each compound was calculated at a signal-to-noise ratio of three based off a low-concentration standard outline in Table 2. Using a 10- $\mu$ L injection of each compound, LOD was converted to an on column mass LOD. Output was entered into Microsoft Excel (Microsoft, Redmond, WA) where datapoints below the respective LOD was given a zero value (E. P. Benton et al. 2015).

Table 2: Precursor, fragment ions, and voltages for imidacloprid and olefin and analytical sensitivity

Compound	Precursor ion ( <i>m/z</i> )	Fragment ion ( <i>m/z</i> )	Declustering Potential (V)	Collision energy (V)	Calibration standard range (ppb)	LOD (ppb)	On-column LOD (pg)
Imidacloprid	256.1	209.1	27	26	336-12.4	0.4 ± 0.1	4.5 ± 1.2
Olefin	209.1	205.1	26	27	321-11.9	1.0 ± 0.3	10.5 ± 3.2

### *HWA Population Counts & Mortality*

Three HWA insect population counts were conducted. The first was collected in the field on May 11, 2017 (pre-treatment), the second on November 20, 2017 (6-months post-treatment), and the last the on April 12, 2018 (11-months post-treatment). Collection was conducted by dividing the canopy into twelve quadrants, three vertically, and four radially. One 10 cm distal branch tip was collected from each quadrant using a Marvin Pole pruner, bagged, labeled, and brought to the lab on ice where it they were stored in the fridge at 4 °C.

During the May 2017 count, strictly egg masses were tallied. For the November 2017 and May 2018 it was decided all stages of life were to be counted. Tallies were made using a stereoscope (Leica Zoom 2000; Leica, Wetzlar, Germany) and probe that was used to elicit movement. When no movement was observed the adelgid was punctured to observe bright hemolymph. If dry, lifeless, or full of black hemolymph, insects were considered dead. Percent mortality was calculated for each group where possible. The use of Abbott's correction formula (Abbott 1925) took the natural mortality rate found in the uncreated control (UTC) and factored this into the mortality of the treated groups.



### *Cofactor Measurement*

In order to account for variation in concentration levels, diameter at breast height (DBH), live crown ratio (LCR), soil moisture and precipitation quantities were recorded when trees were originally identified. Volumetric soil moisture data was measured through use of a time domain reflectrometer (Model CS605, Campbell Scientific 2001). Out of the fifty-six treated trees, thirteen were randomly selected to receive soil moisture readings twice monthly between the months of June 2017 and November 2017. Three readings were taken from inside the dripline of these thirteen trees and then averaged. The daily max, min, and precipitation was recorded to account for variation in mortality rates and was accessed through the nearest NOAA station in Confluence, PA (~14 km miles away). This data can be found in the appendix.

### *Financial Comparison*

Comparisons between soil injections and bark applications were calculated on a per tree basis requiring data points found in Figure 14. The national mean wage of Arborists (\$18.55/hour) was used in the total cost calculation and was taken from the United States Department of Labor website (BLS 2017). Calibration and mixing time were incorporated into this calculation. Product prices differ year to year, between suppliers, with the quantity ordered, between brands, between formulations withing brands, and the amount of active ingredient within formulations. Product prices used for Xytect 75 WSP® and Pentra-bark® - \$3.86/29mL and \$1.65/29mL respectively- were obtained from two suppliers and averaged. Earlier it was mentioned two technicians were used during field treatment. The second technitian ensured the first technitian was adhering to the time required for the proper dosage to be applied to an

individual tree. It is important to note this does not include time maneuvering around the branches of an individual tree or from tree to tree as there was terrain variability of all sites.

$$\text{Treatment Cost (\$)} = \left[ \text{application time (s)} + \left( \frac{\text{calibration time (s)} + \text{mixing time (s)}}{8} \right) * \right. \\ \left. \$ \text{ labor/s} \right] + [(Xytect \text{ applied (mL)} * \$ \text{ Xytect/mL}) + (\text{Pentrabark applied (mL)} * \\ \$ \text{ Pentrabark/mL})]$$

*Formula 1: Financial comparison formula. Cost was simply computed by totaling the labor and product costs on a per tree basis. The formula above shows exactly how these costs were calculated. One calculated, the individual costs were analysed using orthogonal contrasts. To obtain an accurate estimate of how long application took, the length of time required to calibrate and mix the product was incorporated by averaging this over the eight trees that received the same treatment. The average cost of Xytect WSP & Pentra-bark used was \$3.86/29 mL & \$1.65/29 mL and \$0.31/second was used for the cost of labor. "s" = seconds*

### *Statistical Analysis*

To facilitate the analyses of imidacloprid and olefin tissue concentration and biological efficacy, treatments were arranged in a 3x2 factorial fashion, with the addition of the UTC. This allowed for the use of orthogonal contrasts in analyzing the tissue concentrations, mortality rates, and financial efficacy. The use of contrasts was intended to reduce the Type I error rate. Contrasts performed were: 1.) UTC vs all treated trees 2.) soil treatments vs. bark treatments.) low dosage vs. high dosage and 4.) bark treatments vs. bark + Pentra-bark® treatments. Data required a log+1 transformation to obtain a normal distribution while population counts followed a negative binomial distribution that, by nature, transforms data using a log function. Goodness of fit was assessed by chi-square per degree of freedom and all were less than one, indicating a good fit. The PROC GLM and GLIMMIX functions of SAS® were used respectively for analyses (SAS®, Version 9.4, SAS Institute Inc., Cary, NC). DBH and LCR influence on imidacloprid and olefin concentrations was examined through an ANCOVA analysis through the

PROC GLM feature as well. Analyses were performed through JMP® (JMP®, Version Pro 12.2, SAS Institute Inc., Cary, NC). Significance criterion alpha for all tests was 0.05.

## Results

### *Tissue Analysis*

All insecticide treatments produced detectable levels concentrations of imidacloprid and olefin. Means were not significantly affected (Table 4) by the labeled dosage ( $P = 0.1181$ ) or the presence of an adjuvant ( $P = 0.8358$ ). No significant differences were found between bark treatments and soil treatments ( $P = 0.0789$ ). All treatments were significantly different from the UTC in regards to imidacloprid ( $P < 0.0001$ ) and olefin ( $P = 0.0376$ ).

*Table 3: One-way ANOVA with orthogonal contrasts of the mean concentration  $\pm$  SE (standard error) testing effect of untransformed imidacloprid & olefin concentrations. \* indicate significant differences.*

Comparison	N	Imidacloprid (ppb)		Olefin (ppb)	
		Mean $\pm$ SE	Pr >  t	Mean $\pm$ SE	Pr >  t
UTC vs.	8	0.00 $\pm$ 0.00	<.0001*	0.00 $\pm$ 0.00	0.0376*
Treated	48	85.12 $\pm$ 24.33		10.64 $\pm$ 2.93	
Soil vs.	16	161.95 $\pm$ 68.55	0.0789	19.69 $\pm$ 7.78	0.2121
Bark	32	46.70 $\pm$ 8.19		6.12 $\pm$ 9.66	
Low vs.	24	91.18 $\pm$ 45.20	0.1181	13.31 $\pm$ 4.59	0.5013
High Dosage	24	79.06 $\pm$ 19.34		7.97 $\pm$ 3.80	
Bark vs.	16	44.15 $\pm$ 10.46	0.8358	5.59 $\pm$ 2.18	0.7721
Bark + Pentra-bark	16	49.25 $\pm$ 12.94		6.65 $\pm$ 2.69	

## Biological Efficacy

Contrasts of live adults in the spring 2017 ( $P=0.8799$ ), fall 2017( $P=0.0155$ ), and spring 2018 ( $P=0.9817$ ) found no significant differences between groups prior to treatment (spring 17), significant differences between the UTC and treated specimens for the fall of 2017, and no differences in the spring of 2018 (Table 5). Meanwhile, the mortality comparisons between the fall 2017 and spring 2018 counts reveal: that morality across all groups in the fall was high (Figure 15), there was a significant difference between morality in the soil and bark treated groups in the fall of 17 ( $P = 0.0384$ , Table 6), morality increased from the fall to spring across all groups yielding no difference between the soil/bark treatments in the spring ( $P = 0.0855$ ), and the UTC mortality decreased from fall to spring.

*Table 4: One-way ANOVA with orthogonal contrasts of live adults in the spring 2017 (Time = 0 months), fall 2017 (Time = 6 months), & spring 2018 (Time = 11 months) Findings include no significant differences were found pretreatment (spring 17), significant differences between the UTC and treated specimens fall 2017), and no differences in the spring of 2018.*

Comparison	N	Spring 17		Fall 17		Spring 18	
		Mean $\pm$ Error	Pr >  t	Mean $\pm$ Error	Pr >  t	Mean $\pm$ Error	Pr >  t
UTC vs.	8	8.62 $\pm$ 4.86	0.8799	99.12 $\pm$ 45.22	0.0155*	0.62 $\pm$ 0.26	0.9817
Treated	48	15.12 $\pm$ 5.74		19.81 $\pm$ 5.47		2.33 $\pm$ 1.32	
Soil vs.	16	18.78 $\pm$ 12.79	0.5751	36.33 $\pm$ 11.91	0.0216*	1.05 $\pm$ 0.73	0.9778
Bark	32	12.93 $\pm$ 5.24		9.90 $\pm$ 4.32		3.10 $\pm$ 2.07	
Low vs.	24	23.58 $\pm$ 11.00	0.0241*	22.95 $\pm$ 7.78	0.2428	4.00 $\pm$ 2.57	0.9861
High Dosage	24	6.66 $\pm$ 2.73		16.66 $\pm$ 7.81		0.66 $\pm$ 0.54	
Bark vs.	16	14.71 $\pm$ 10.41	0.5213	8.85 $\pm$ 3.82	0.9455	0.07 $\pm$ 0.07	0.9747
Bark + Pentra-bark	16	11.37 $\pm$ 4.12		10.81 $\pm$ 7.52		5.57 $\pm$ 3.81	

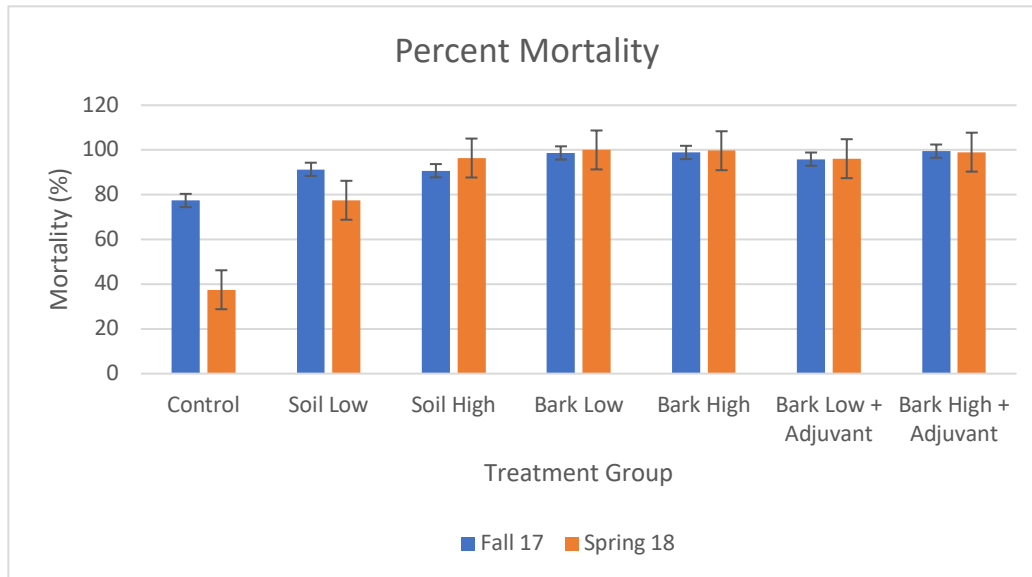


Figure 16: Percent mortality for each count where the total number of adelgid across all life stages were collected. The use of Abbott's formula adjusts for the percent mortality experienced by UTC (Abbott 1925). Results show mortality decreased from the fall to spring in the UTC, while the mortality between the soil and bark treatments were significantly different in the fall. Come spring, these differences between the soil and bark treatments was reduced.

Table 5: Mortality contrasts show significant differences in fall mortality between the UTC control and the treated groups. Differences occur between the untreated and treated groups for both counts, and between soil/bark treatments for the fall of 2017. This difference no longer exists by the spring.

Comparison	N	Fall 17		Spring 18	
		Mean $\pm$ Error	Pr >  t	Mean $\pm$ Error	Pr >  t
UTC vs. Treated	8 48	20.74 $\pm$ 0.19 80.28 $\pm$ 69.28	<.0001*	37.50 $\pm$ 12.50 80.97 $\pm$ 7.75	0.0025*
Soil vs. Bark	16 32	57.60 $\pm$ 0.19 91.62 $\pm$ 3.92	0.0384*	48.8 $\pm$ 21.33 97.05 $\pm$ 1.22	0.0855
Low vs. High Dosage	24 24	77.76 $\pm$ 8.40 82.79 $\pm$ 35.00	0.7490	65.66 $\pm$ 14.81 96.28 $\pm$ 2.39	0.2365
Bark vs. Bark + Pentra-bark	16 16	94.21 $\pm$ 2.76 89.03 $\pm$ 7.41	0.7803	99.61 $\pm$ 0.33 94.4 $\pm$ 2.280	0.8987

### *Financial Efficacy*

Significant cost differences exist (Table 7) between the UTC versus all insecticide treatments ( $P = 0.0015$ ), and insecticide treatments of the low and high dosages ( $P = 0.0004$ ) in which the high dose was 32% higher in cost. No differences of cost were found between soil versus bark treatments (0.1194) or bark versus bark + Pentra-bark® (0.1009).

*Table 5: Financial contrast found differences between UTC/all treatments and low/high dosages while no differences exist between the soil/bark treatments and bark/bark + Pentra-bark treatments.*

Comparison	N	Mean Cost (\$) $\pm$ Error	Pr >  t
UTC vs.	8	0.00 $\pm$ 0.00	0.0015*
Treated	48	1.79 $\pm$ 0.07	
Soil vs.	16	1.77 $\pm$ 0.15	0.1194
Bark	32	1.80 $\pm$ 0.08	
Low vs.	24	1.55 $\pm$ 0.09	0.0004*
High Dosage	24	2.04 $\pm$ 0.10	
Bark vs.	16	1.68 $\pm$ 0.10	0.1009
Bark + Pentra-bark.	16	1.91 $\pm$ 0.13	

### *Cofactors*

Soil moisture appeared to be adequate across the site for plant growth in the silt loams of the site. In silt loams, water is available when percentages are above 14% and all measurements were above this level. This is explained, in part, by the amount of rainfall experienced in the area (Appendix). From these averages, a lack of available water did not inhibit water uptake and, in turn, imidacloprid translocation (Table 8 and Figure 16). The average monthly minimum temperatures ranged from 1.11 °C to -10.98 °C between the coldest months of November through March (Appendix).

Table 7: Percent soil moisture was collected twice monthly with TDR on a sub-sample of trees and averaged.

Month (2017)	% Soil Moisture
June	19.65
July	22.26
August	16.80
September	14.80
October	23.93
November	23.86

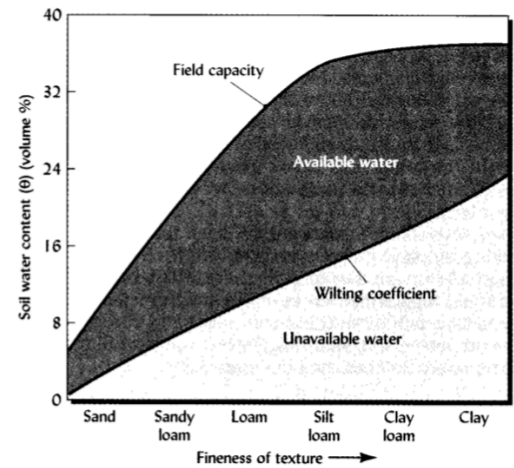


Figure 17: Monthly averages found in Table 8, fell within the available water band for the silt loams of the site. Water up-take and imidacloprid translocation were not of detriment to the product. Graph from Bradley & Weil 1999.

DBH was found to have an effect on the tissue concentrations of imidacloprid ( $P = 0.0167$ ,  $R^2 = 0.4452$ , Figure 18). Regression equations for the treatments are: soil low  $= 4.87 - 0.05 \cdot \text{DBH}$ , soil high  $= 7.34 - 0.22 \cdot \text{DBH}$ , bark low  $= 6.34 - 0.23 \cdot \text{DBH}$ , bark high  $= 6.02 - 0.24 \cdot \text{DBH}$ , bark low + pentra-bark  $= 5.71 - 0.24 \cdot \text{DBH}$ , and bark high + pentra-bark  $= 4.24 + 0.00 \cdot \text{DBH}$ . Meanwhile DBH had no effect on olefin concentrations ( $P = 0.1580$ ). LCR was not found to have an effect on the concentration of either compound tested; imidacloprid ( $P = 0.2068$ ) or olefin ( $P = 0.1224$ ).

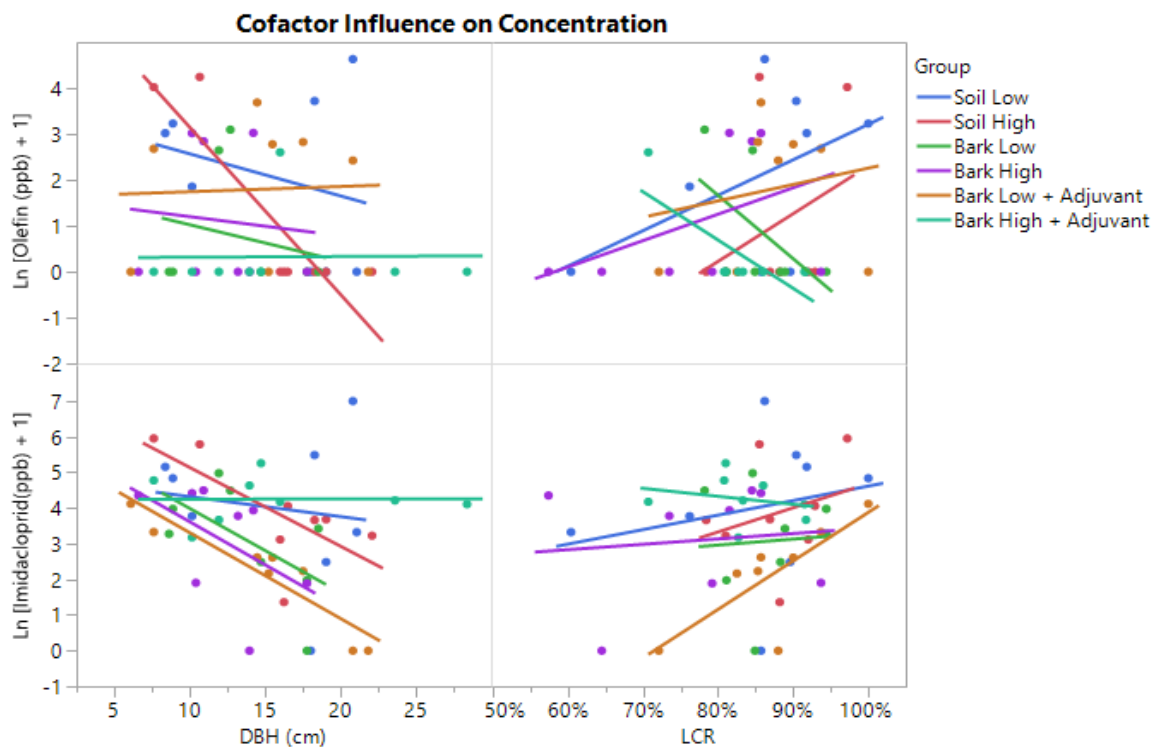


Figure 18: Tissue concentration and cofactor relationships. DBH was the only cofactor to influence the concentration of either compound (DBH,  $P=0.0167$ ). As DBH increases, tissue concentrations of imidacloprid are reduced, as noted in several studies.

## Discussion

This study was designed to investigate whether basal bark applications of imidacloprid can be used to treat HWA rather than other application methods. Before proceeding, it is important to address variability within the data enumerated in Table 3 and the Appendix. Significant variability existed among several treatments, in particular the soil low treatment. This was due to an outlier three times the standard deviation. Variability in the bark treatments may partly exist due to the inability of equipment to maintain the calibration established as mentioned earlier.

Results suggest imidacloprid basal bark applications may be added as one more tool in an IPM technician's arsenal when treating for HWA. This study found basal bark applications



produce imidacloprid and olefin tissue concentrations similar to those associated with soil injections. In addition, concentrations were similar to those found in other studies, despite different application methods (Turcotte 2015, Cowles and Lagalante 2009, Benton et al. 2016). In one instance, where bark applications were also used, imidacloprid concentrations obtained in this study appear to be higher (McCullough et al. 2011). Bark penetration was not influenced by labeled dosage or the presence of Pentra-bark®. Findings also suggest HWA mortality of bark applications is comparable to soil injections and may produce higher levels of mortality in a shorter timespan, after which it levels off.

These results are not surprising for many reasons. Imidacloprid is one of several neonicotinoids that are water soluble and have been proven (as a class of insecticides) to work as a basal bark spray providing sufficient insecticide concentrations for bark penetration and sufficient insect mortality (Cowles 2010). It is the water solubility that, at least in part, dictates the penetrating abilities of a chemical. The addition of an adjuvant in this study was not found to increase the concentrations of imidacloprid or olefin, nor lead to greater mortality. Results of the adjuvant efficacy corroborate the findings of both Cowles (2010) and McCullough (2011), suggesting that there is little reason to Pentra-bark® adjuvant in basal bark applications for HWA. This is not to say it does not work with other pesticides for other pests. On the other hand, bark treatments + Pentra-bark® were not any more expensive than bark treatments lacking Pentra-bark® or their soil injection counterparts.

The cofactors observed, DBH & LCR, were only observed to influence imidacloprid concentrations. As other studies have noted (Turcotte et al. 2016, Ford et al. 2010) the concentration decreases as DBH increases due to the fact water usage is exponentially related to

DBH. This means smaller trees use proportionally less water than their larger counterparts (Ford et al. 2010). Future researchers may wish to investigate if dose should be adjusted with DBH.

Mortality was found to be significantly higher in the bark treatments over the soil treatments during the fall of 2017 the, but not different in the spring of 2018. This difference was found despite the lack of significant difference in concentrations of imidacloprid or olefin in the tissue samples taken in the fall. The quick mortality by basal bark applications was unexpected, however, it is plausible that the insecticide can enter the xylem more quickly as it is not as dependant on soil moisture for uptake (Norris 1967). These results vary slightly from previous research conducted by Faulkenberry (2012) whose study found that at approximately six months post treatment, mortality between imidacloprid bark applications and soil drenches did not significantly differ. It should be noted that the Faulkenberry reseach did not utilize a correction for natural mortality such as Abbott's correction formula. Future research could investigate whether imidacloprid bark applications move more quickly into the xylem by sampling tissues throughout the growing season. Parker (1998) established that HWA coldhardiness diminishes over the cold months as March approaches and that prolonged cold temperatures of -20° C can impact HWA populations (Cost et al. 2008). The monthly mean minimum temperature reached lowest temperatures in the month of January, with an average of -10°C. These temperatures could have contributed to the increase of mortality across the treatments.

While the cost for the bark applications of imidacloprid was not found to differ from that of soil applications, having the bark application in the IPM toolbox allows additional flexibility. Additionally, since there was no difference between the high and low rate of imidacloprid in terms of tissue conentration and mortality, using a lower does could reduce cost by approximately 32%.

These savings could potentially be reduced further. Norris (1967) suggests that bark applications may require 1/10<sup>th</sup> to 1/15<sup>th</sup> the amount of product compared to other application methods. Similarly, mixing imidacloprid with less water could result in higher tissue concentrations, as during application, some of the insecticidal mixture runs down the bark and onto the soil. By increasing the concentration of the imidacloprid/water mixture, perhaps less water can be used, tissue concentrations may be higher, application times could be shorter, and applications less costly.

The data seems to suggest, if the bark application method was added to the product label, arborists could have a product that is comparable to the soil injections commonly used in terms of insecticidal concentrations, insect mortality, and cost. If approved, bark applications are another application method that yield comparable results with some additional benefits, making the use of this method a decision of practicality. In the meantime, more details about this method can be probed.

Researchers may also wish to test on stands with a higher HWA population. The trees in this study had a relatively low level of HWA infestation. Population surveys had not been conducted previously. With no quantitative data, prior infestation rates are unknown. If significant populations had existed at one point, they most likely were reduced by several winters with prolonged periods of below freezing temperatures. Thirdly, the long-term effects of bark treatments should be studied in regards to longevity, tissue concentration, and mortality rates. With such a high natural mortality rate present in the UTC and such a small number of living insects, it may be advisable to conduct research on stands with a more dense population to get a clearer picture of just how this application method affects pest populations. Lastly, with a wide

variety of backpack sprayers available for purchase, investigation into reliable models, brands, or measurement equipment should be pursued for scientific and commercial use.

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## Appendix

### Raw Data

Group	Diam (cm)	HT (m)	LCR	olefin (ppb)	imidacloprid (ppb)	Total Cost (\$)
Untreated Control #1	19.30	9.75	90.63%	0.0	0.0	-
Untreated Control #2	13.46	10.73	88.35%	0.0	0.0	-
Untreated Control #3	17.27	5.30	76.44%	0.0	0.0	-
Untreated Control #4	7.62	3.57	87.18%	0.0	0.0	-
Untreated Control #5	12.19	6.49	82.16%	0.0	0.0	-
Untreated Control #6	14.48	8.38	87.27%	0.0	0.0	-
Untreated Control #7	10.16	5.15	94.08%	0.0	0.0	-
Untreated Control #8	9.91	5.82	89.01%	0.0	0.0	-
Soil Injection Low #1	20.83	8.84	86.21%	101.9	1088.6	1.67
Soil Injection Low #2	18.03	10.64	85.67%	0.0	0.0	1.47
Soil Injection Low #3	19.05	10.52	89.57%	0.0	11.0	1.48
Soil Injection Low #4	21.08	7.16	60.43%	0.0	26.7	1.73
Soil Injection Low #5	8.38	4.08	91.79%	19.5	171.6	0.84
Soil Injection Low #6	8.89	3.99	100.00%	24.3	124.6	0.95
Soil Injection Low #7	18.29	8.53	90.36%	40.3	239.1	1.43
Soil Injection Low #8	10.16	8.32	76.19%	5.4	42.4	0.95
Soil Injection High #1	22.10	10.42	80.99%	0.0	24.1	2.83
Soil Injection High #2	19.05	10.73	86.93%	0.0	38.7	2.51
Soil Injection High #3	10.67	5.06	85.54%	68.8	323.7	2.20
Soil Injection High #4	16.51	8.63	92.93%	0.0	56.7	2.49
Soil Injection High #5	16.00	8.05	92.05%	0.0	21.5	2.44
Soil Injection High #6	16.26	10.82	88.17%	0.0	2.9	1.80
Soil Injection High #7	7.62	5.52	97.24%	54.9	381.8	1.24
Soil Injection High #8	18.29	10.15	78.38%	0.0	37.9	2.41
Bark Low #1	18.54	9.05	88.89%	0.0	29.6	1.93
Bark Low #2	8.89	4.94	94.44%	0.0	52.2	1.15
Bark Low #3	17.78	9.11	84.95%	0.0	0.0	1.66
Bark Low #4	14.73	10.45	88.34%	0.0	11.0	1.52
Bark Low #5	12.70	8.69	78.25%	21.1	88.0	1.40
Bark Low #6	8.64	2.74	94.44%	0.0	25.4	1.14
Bark Low #7	11.94	7.89	84.56%	13.1	143.6	1.36
Bark Low #8	17.78	11.31	81.13%	0.0	6.2	1.62
Bark High #1	17.78	4.69	79.22%	0.0	5.6	2.64
Bark High #2	6.60	4.30	57.45%	0.0	76.4	1.17
Bark High #3	13.97	4.72	64.52%	0.0	0.0	2.19
Bark High #4	10.92	9.85	84.52%	16.2	88.3	1.85
Bark High #5	13.21	10.36	73.53%	0.0	42.7	2.01
Bark High #6	10.41	5.76	93.65%	0.0	5.7	1.32
Bark High #7	10.16	6.40	85.71%	19.5	81.7	1.69
Bark High #8	14.22	8.26	81.55%	19.6	50.1	2.24
Bark High + Adj. #1	23.62	10.42	83.33%	10.3	66.5	3.00
Bark High + Adj. #2	7.62	3.17	80.77%	13.6	117.1	1.20
Bark High + Adj. #3	16.00	7.07	70.69%	0.0	63.9	2.05
Bark High + Adj. #4	10.16	7.92	82.69%	0.0	22.9	1.86
Bark High + Adj. #5	14.73	9.97	81.04%	39.0	190.2	2.28
Bark High + Adj. #6	11.94	6.61	91.71%	0.0	38.1	1.79
Bark High + Adj. #7	13.97	6.55	86.05%	15.1	101.4	1.89
Bark High + Adj. #8	11.20	8.99	91.53%	15.9	59.6	2.74
Bark Low + Adj. #1	20.83	10.45	88.05%	0.0	0.0	2.25
Bark Low + Adj. #2	7.62	4.85	93.71%	0.0	26.8	1.07
Bark Low + Adj. #3	6.10	3.23	100.00%	12.5	60.3	0.94
Bark Low + Adj. #4	15.24	8.72	82.52%	0.0	7.7	1.76
Bark Low + Adj. #5	14.48	10.00	85.67%	0.0	12.7	1.71
Bark Low + Adj. #6	21.84	10.94	72.14%	0.0	0.0	2.44
Bark Low + Adj. #7	15.49	2.44	90.00%	0.0	12.6	1.76
Bark Low + Adj. #8	17.53	9.51	85.26%	0.0	8.3	1.98

### Treatment & Weather Data Means

Group	N	Imidacloprid (ppb)	Olefin (ppb)	DBH (cm)	LCR (%)	Cost (\$)
UTC	8	0.0	0.0	13.04 ± 1.38	86.75 ± 1.90	0.00 ± 0.00
Soil Low	8	213.0 ± 128.6	23.9 ± 34.6	15.58 ± 5.46	85.03 ± 4.23	0.61 ± 0.03
Soil High	8	110.9 ± 53.3	15.4 ± 28.8	15.81 ± 4.63	87.76 ± 2.20	0.84 ± 0.07
Bark Low	8	44.5 ± 17.3	4.2 ± 8.1	13.87 ± 3.97	86.85 ± 2.05	0.84 ± 0.03
Bark High	8	43.8 ± 12.9	6.9 ± 9.5	12.16 ± 3.37	77.50 ± 4.19	0.78 ± 0.06
Bark Low + Pentra-Bark	8	25.0 ± 8.34	10.4 ± 12.8	14.89 ± 5.62	87.16 ± 2.89	1.06 ± 0.09
Bark High + Pentra-Bark	8	84.7 ± 21.5	1.7 ± 4.7	15.8 ± 6.94	83.46 ± 2.37	0.99 ± 0.10

Month	Year	Max (°C)	Min (°C)	Rain melted, snow, etc. (cm)	Snow, Ice pellets, hail (cm)	Snow, Ice Pellets, Hail, Ice on Ground (cm)
5	2017	21.34	9.14	0.09	0.00	0.00
6	2017	26.54	13.22	0.08	0.00	0.00
7	2017	27.97	16.56	0.15	0.00	0.00
8	2017	26.43	13.82	0.06	0.00	0.00
9	2017	24.09	11.28	0.04	0.00	0.00
10	2017	20.93	6.49	0.08	0.00	0.00
11	2017	10.00	-2.07	0.05	0.00	0.00
12	2017	3.51	-6.01	0.02	0.15	0.28
1	2018	1.15	-10.98	0.04	0.21	0.76
2	2018	10.02	-3.58	0.13	0.17	0.41
3	2018	7.44	-3.69	0.19	0.19	0.35
4	2018	14.44	1.11	1.82	1.18	1.57

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Figure #	Page #	UGA #	Subject	Citation
1	4	-	Global Hemlock Distribution	Nathan Havill, USDA Forest Service, Northern Research Station
2	6	-	Global HWA Distribution	Carole Cheah, Connecticut Agricultural Experiment Station
3	8	-	North American Lifecycle	Jeffrey Ward, Connecticut Agricultural Experiment Station
4a	11	5018078	Eggs	Pennsylvania Department of Conservation and Natural Resources, Bugwood.org
4b	11	5018073	Crawler	Pennsylvania Department of Conservation and Natural Resources, Bugwood.org
4c	11	5449457	Crawler Closeup	Kelly Oten, North Carolina Forest Service, Bugwood.org
4d	11	5449456	Nymph Closeup	Kelly Oten, North Carolina Forest Service, Bugwood.org
4e	11	5473217	Multiple Nymphs	Ashley Lamb, Virginia Polytechnic Institute and State University, Bugwood.org
4f	11		Nymph Stylet	
4g	11	5476127	Adults	USDA Forest Service Southern Research Station, Bugwood.org
4h	11	5449453	Adult Electron Microscope	Kelly Oten, North Carolina Forest Service, Bugwood.org
4i	11	-	Winged Sexupare	Nathan Havill, USDA Forest Service, Northern Research Station
4j	11	-	Fundatrix	Nathan Havill, USDA Forest Service, Northern Research Station
5	12	-	Complete Lifecycle	Nathan Havill, USDA Forest Service, Northern Research Station
6a	16	1276009	Sassajiscymnus tsugae	Carole Cheah, Connecticut Agricultural Experiment Station, Bugwood.org
6b	16	5017090	Scymnus sinuanodulus	Pennsylvania Department of Conservation and Natural Resources, Bugwood.org
6c	16	1276021	Laricobius nigrinus	Ashley Lamb, Virginia Polytechnic Institute and State University, Bugwood.org
7	21	-	Metabolic Pathway	F. J. Placke
8	25	-	Chemical Properties	Matthew Fossen, California Department of Pesticide Regulation
9	27	-	Toxicity Ratings	Matthew Fossen, California Department of Pesticide Regulation
10a	29	-	Height Limitations	Matt Quinterno
10b	29	-	Spray Drift	Matt Quinterno
10c	29	796066	Pesticide Drift	Larry R. Barber, USDA Forest Service, Bugwood.org
11a	30	-	Soil Drench	Matt Quinterno
11b	30	-	Calibrated Soil Probe	Rainbow Treecare Scientific
11c	30	1344053	Kioritz	Great Smoky Mountains National Park Resource Management, USDI National Park Service, Bugwood.org
11d	30	-	Motorized Backpack	Rainbow Treecare Scientific
11e	30	-	Bottle Pump	Matt Quinterno
12a	31	5005031	Mauget Capsule	Ronald F. Billings, Texas A&M Forest Service, Bugwood.org
12b	31	-	Arborjet Injector	Arborjet
12c	31	-	Wedgel Injector	Arborsystems
13	32	-	Backpack Sprayer	Matt Quinterno
14	39	-	Application Equipment	Matt Quinterno

